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1258332

December 18, 2012

Christina Progess
USEPA; Region 8
1595 Wynkoop Street (8EPR-SR)
Denver, CO 80202-1129

Document ID #: 2021-12182012-1

Dear Ms. Progess:

EPA CONTRACT NUMBER EP-W-10-033
TASK ORDER NUMBER 2021
QA SUPPORT FOR RI/FS AT THE LIBBY ASBESTOS SITE OU3

Enclosed please find the Summary On-site Evaluation Report for the on-site evaluation performed on November 19, 2012 at Fort Environmental Laboratories, Inc. (FEL) in Stillwater, OK. This report and the accompanying checklist are deliverables under Task 2 of the subject Task Order.

If you have any questions, please feel free to contact me.

Sincerely,

Timothy Vonnahme

Timothy L. Vonnahme
Audit Group Manager, QATS Program

cc: Shari Myer, EPA-ASB QATS Project Officer
Administrative Contracting Officer (Letter only)
Audit Group Files

REPORT

FOR

TASK ORDER NUMBER 2021
QA SUPPORT FOR RI/FS AT THE LIBBY ASBESTOS SITE OU3

SUMMARY ON-SITE EVALUATION REPORT

Fort Environmental Laboratories, Inc. - Stillwater, OK (FEL)

Amphibian Complete Metamorphosis Exposure Study
FEL Study No. GOLD03-00277

Prepared by:

Michael P. Lenkauskas

The Data Auditing Group
Quality Assurance Technical Support Program
Shaw Environmental, Inc.
2700 Chandler Avenue
Las Vegas, Nevada 89120

December 17, 2012

QATS Contract Number: EP-W-10-033

Prepared for:

Christina Progess
USEPA Region 8

Through:

Analytical Services Branch
U.S. Environmental Protection Agency
Washington, D.C. 20460

OFFICE OF SUPERFUND REMEDIATION AND TECHNOLOGY INNOVATION
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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LABORATORY INFORMATION AND ON-SITE EVALUATION SCOPE

This report summarizes the findings of an on-site laboratory evaluation of Fort Environmental Laboratories, Inc. (FEL) in Stillwater, Oklahoma conducted on November 19, 2012. The on-site visit involved a compliance assessment of laboratory procedures to the protocol, Amphibian Complete Metamorphosis Exposure Study (FEL Study No. GOLD03-00277). Shaw Environmental, Inc. Quality Assurance Technical Support (QATS) staff participation in the evaluation and subsequent preparation of this report was performed under Tasks 1 and 2 of Task Order (TO) 2021.

Detailed information regarding the subject laboratory is as follows:

Date(s) of On-site:	**November 19, 2012**
Laboratory:	**Fort Environmental Laboratories (FEL), Inc.** **515 South Duncan Street** **Stillwater, OK 74074** **(405) 624-6771**
President:	**Douglas J. Fort, Ph.D.**
On-site evaluation Team	
US EPA:	**Christina Progess, Libby OU3 Project Manager**
Shaw QATS:	**Michael Lenkauskas, CQA, Senior Auditor**
Golder Associates:	**Sue Robinson, Senior Toxicologist**
Remedium Group:	**Robert J. Medler, Director**

The on-site evaluation team, comprised of United States Environmental Protection Agency (USEPA) Region 8, Golder Associates, Remedium Group, and Shaw Environmental, Inc. QATS personnel, performed the technical and evidentiary aspects of the evaluation. The technical part of the evaluation involved a thorough evaluation of the laboratory's procedures as applied to the study, including the shipping and receiving of test organisms, reference sediments, and test sediments and the monitoring (physical and chemical) of the exposure systems. The evidentiary part of the audit included a review of the laboratory's record keeping practices for shipping and receiving of test materials and test organisms; exposure system preparation; analytical measurements; the availability of written procedures; and the presence of a viable quality assurance/quality control (QA/QC) program. An EPA-approved Libby OU3 Amphibian Toxicity Study On-Site Evaluation Checklist (completed by the QATS staff during the course of the audit) is included as an attachment to this report (EPA only).

EXECUTIVE SUMMARY

Fort Environmental Laboratories, Inc. (FEL) was contracted by Golder Associates to conduct a toxicological study to examine the effects of Libby Amphibole (LA) asbestos on the complete metamorphosis of ranid amphibians. The results of the study will be used to support the evaluation of potential ecological risk at the Libby Asbestos Superfund Site in Libby, Montana. The study is being conducted in accordance with the specifications identified in the Phase V-B Sampling and Analysis Plan (SAP), FEL Quality Assurance Management Plan (QAMP), all relevant facility standard operating procedures (SOPs), and the study protocol. This study involves a complete amphibian metamorphosis assay in which ranid larvae, beginning at Gosner Stage 20, are exposed to LA. The general experimental design entails exposing the test organisms to contaminated soil collected from the Libby Superfund Site and two controls, inert sand and reference sediment. The primary endpoints of the study are survival, developmental stage, time to metamorphosis for each test organism, median time to metamorphosis for each replicate, metamorphic count, external and internal malformations, whole body weight, and snout-vent length of each surviving test organism. The blood, head, and torso of the larvae will also be preserved for possible further study, if deemed necessary, based on the results of this study.

On November 19, 2012, the evaluation team performed an on-site laboratory evaluation of FEL on day 55 of the scheduled 90 day study. This on-site evaluation is required by Section 2.7 of the SAP/QAPP for Operable Unit 3 of the Libby Superfund Site (Phase V, Part B: 2012 Ecological Investigations). Prior to arriving on-site, QATS personnel reviewed all pertinent FEL quality documents (i.e., QAMP and SOPs), the applicable Libby Superfund Site SAP/QAPP, and the study protocol from which QATS developed an on-site evaluation checklist that was completed during the evaluation. Upon arrival at FEL the laboratory staff and the evaluation team participated in a briefing during which the evaluation team outlined the scope of the evaluation and FEL staff provided an update of study progress, which was initiated on September 26, 2012. The evaluation involved an assessment of the equipment, applied procedures, records, and data from FEL for six aspects of the study: Study Design, Water Quality and Sediment Analyses, Test Species and Life Stage, In-Life Measurements and Observations, Study Termination, and Quality Assurance/Quality Control (QA/QC). One process observation was identified related to failure to analyze the laboratory control (inert sand) and reference sediment for the presence of LA prior to initiation of the in-life study. This was discussed with the laboratory at the debriefing. Note that, on November 28, 2012, FEL provided a protocol change to Golder Associates which addressed this issue.

Overall the on-site evaluation revealed FEL to have sufficient facilities, equipment, and staff to meet the requirements described in the amphibian toxicity study protocol. The staff interviewed appeared to have sufficient training and understanding of the protocol requirements, and all staff and management were cooperative, readily answered questions by the evaluation team, and were responsive to observations made.

ON-SITE EVALUATION COMMENTS AND OBSERVATIONS

The evaluation of the FEL Amphibian Complete Metamorphosis Exposure Study was performed by the evaluation team on November 19, 2012, which was day 55 of the scheduled 90-day study. The evaluation involved an assessment of the equipment, applied procedures, records, and data from FEL for six aspects of the study: Study Design, Water Quality and Sediment Analyses, Test Species and Life Stage, In-Life Measurements and Observations, Study Termination, and QA/QC.

Study Design

The study design consisted of the following three exposure systems:

Exposure 1 – Synthetic sediment (laboratory control) plus laboratory water
Exposure 2 – Reference (uncontaminated) field sediment plus laboratory water
Exposure 3 – Contaminated field sediment (≈5% LA) plus laboratory water

Each of the three exposure systems consisted of four replicate exposure chambers (2.5 gallon aquaria), 12 chambers in all, each of which contained 20 test organisms. Each of the aquaria was fitted with standpipes to maintain a tank volume of 6 liters, a heater to maintain proper temperature, and a flow-through system of approximately 12 milliliters a minute for water renewal. Fluorescent lighting was used to simulate photoperiods of 12 hours of light and 12 hours of dark. The evaluation team found the study design to be as described in the study protocol, with no deviations observed.

Water Quality and Sediment Analyses

The test substance (Exposure 3) was natural LA contained within sediment collected from Carney Creek, which is within the Libby Superfund Site. Prior to shipment of the test sediment to FEL, an aliquot was tested by EMSL (Libby, MT) in accordance with the SOP SRC-Libby-03 (PLM-VE). The percentage of LA was determined to be approximately 5%. Additional aliquots of the test sediment were also shipped to Energy Labs (Billings, MT) where the samples were analyzed for pesticides, herbicides, total organic carbon, hydrocarbons (gas and diesel), mercury, total metals, moisture content, pH, pesticides, Aroclors, semi-volatile organics, acid volatile sulfide, and ammonia. Both the laboratory control sediment (inert sand) and the reference sediment, which was collected from a nearby pond, were analyzed for total metals, polyaromatic hydrocarbons (PAHs), and Aroclors.

Dilution and laboratory water was prepared by passing tap water through a four-filter system to remove suspended solids, chlorine, ammonia, high molecular weight organics, and carbons particles. Water quality characteristics are monitored bi-monthly for pH, dissolved oxygen, conductivity, hardness, alkalinity, ammonia, residual oxidants, and at least annually for iodide, PAHs, pesticides and metals.

The evaluation team reviewed the laboratory results from the analyses of the test, control, and reference sediments and the logbook containing the bi-monthly and annual results from the testing of the laboratory control water. The results of all non-asbestos analyses were within the required limits. One observation was identified by the evaluation team concerning the determination of LA in the laboratory control and reference sediments.

1. **Observation:** Neither the laboratory control (inert sand) nor the reference sediment had been analyzed for the presence or absence of LA prior to initiation of the in-life study, as described in Section 8.1 of the study protocol.

   Recommended Corrective Action: Amend the study protocol to ensure aliquots of both the laboratory control and reference sediments are collected from each replicate and composited for LA analysis by PLM-VE (SOP SRC-Libby-03).

   Note: On November 28, 2012, a Document Amendment Form was received from FEL through Golder Associates formalizing the change in the study protocol as described above. A copy the Document Amendment Form is provided as Attachment 1.

Test Species and Life Stage

The ranid species selcted for this study were Southern Leopard Frogs, the embryos of which were provided by the C.A. Sullivan Company. In accordance with study protocol, test organism embryo, which originated from a single hatch, were kept at 22-24°C to allow for hatching and then separated into small density tanks where they were kept at 20-23°C until they reached the specified developmental stage (Gosner Stage 20). Upon reaching Gosner Stage 20, which was determined using a binocular dissection microscope, the test organisms were transferred to a holding tank containing 100% dilution water and then randomly distributed to each of the exposure systems with 20 test organisms per replicate or 80 per exposure system. To verify the developmental stage at initiation of the in-life study, five randomly selected Gosner Stage 20 test organisms were preserved and archived. Although the evaluation team was not present during this stage of the study, a tour of the tanks used for hatching and holding was provided, and the study records were reviewed for compliance with the study protocol. No deviations from the study protocol were observed.

In-life Measurements and Observations

Each of the three exposure systems (control, reference, and test) were maintained in separate, covered containers, with each of the replicates monitored daily for pH and dissolved oxygen, and three times per week for light intensity. Total hardness, alkalinity, conductivity, total residual oxidants, and ammonia were measured at beginning, semi-monthly, and will be measured at the conclusion of the in-life study. Throughout the in-life element of the study, each of the four replicates of each exposure system were observed throughout the day to monitor for abnormal behavior and/or mortalities of test organisms, none of which had been observed to-date. Test organisms were fed boiled lettuce, which was weighed prior to feeding and upon removal, to determine test organism consumption. Any signs of abnormal behavior along with food consumption or mortalities were recorded in the study record. Note that, at the time of the evaluation, there had been no mortalities and no abnormalities observed. Since the endpoint of the in-life study is metamorphosis (Gosner Stage 46), daily observations of the developmental stage of test organisms were made and recorded in the study record. At the time of the evaluation, none of the test organisms had reached metamorphosis; however, laboratory staff expected this to occur at any time. All measurements and observations were recorded in the logbooks and study records reviewed by the evaluation team as were the calibrations records for each of the monitoring devices. No deviations from the study protocol were observed.

Study Termination

Study termination is reached when all test organisms have reach metamorphosis (Gosner Stage 46), at which time test organisms are removed from the exposure tanks. After removal, test organisms are anesthetized, weighed to the nearest milligram, digitally photographed to determine snout vent length (SVL) and record external malformations, and plasma collected for potential future use. Once these data have been collected, the test organisms are euthanized and necropsied for possible histopathological examination. At the time of the evaluation, the study was in the in-life stage, and termination, which is scheduled for no later than December 24, 2012, had not been reached. However, the FEL staff did provide a demonstration of the digitizing software that would be used to measure SVL and document external malformation observations. No deviations from the study protocol were observed.

Quality Assurance/Quality Control

Prior to performing the on-site evaluation, QATS personnel reviewed the applicable FEL SOPs and QAMP to determine whether these documents included sufficient controls to ensure the amphibian study would be performed in a manner consistent with the requirements of the Libby Superfund Site SAP/QAPP and study protocol. While on-site, the evaluation team reviewed the study records, interviewed the Quality Assurance Unit Manager (QAUM), and evaluated whether the QA/QC elements described in the applicable quality documents had been and/or will be applied to this study. The evaluation team evaluated the study records and found them to be complete, accurate, and, with one exception (pre-testing of the laboratory control and reference test substances for LA), compliant with both the external and internal requirements in the applicable quality documents. In addition to maintaining the study records, the evaluation team determined the QAUM also performs various quality checks throughout the course of all studies, including, but not limited, to a protocol review; Technical System Audits (TSAs) performed at study initiation, conclusion, and one additional time during each study; and a thorough, documented review of the study records and draft and final reports. The evaluation team found FEL's quality systems to be robust and implemented at each stage of the study.

CONCLUSIONS

The on-site evaluation of FEL performed on November 19, 2012, included an assessment of the laboratory facility and equipment, procedures, QA/QC program, and the completeness and accuracy of documentation compiled for the Amphibian Complete Metamorphosis Exposure Study. The evaluation revealed FEL to have sufficient facilities, equipment, and staff to effectively perform the procedures described in the study protocol and Libby Superfund SAP/QAPP. In addition, the evaluation team determined FEL to be in compliance with the requirements set forth in these documents with one exception. This observation, which was related to failure to perform pre-testing of the laboratory control and reference test substances for LA, was addressed through a Document Amendment Form received on November 28, 2012, which is provided as Attachment 1 to this report. The staff interviewed appeared to have sufficient training and understanding of the protocol requirements, were cooperative, readily answered questions by the evaluation team, and were responsive to the observations made by the evaluation team.

ATTACHMENT 1

FEL Document Amendment Form

FEL

DOCUMENT AMENDMENT FORM
Fort Environmental Laboratories

Document or Study Title: Amphibian Complete Metamorphosis Exposure Study

Amendment Number: 2 | **Document ID Number**: GOLD03-1

Submitted By: Douglas J. Fort | **Date**: 10/20/2012

Amendment Relating To: GOLD03-00277

[X] Protocol **[ ] Study Plan** **[ ] QAPP** **[ ] QAMP** **[ ] SOP**

[ ] Other (describe):

Original Specifications: Pages 6-7, Section 8.1. Test Substance

"LA will be measured again at the conclusion of the treatment exposure to verify exposure concentrations. In the event that TP-TOE2 contains lower levels of LA based on analyses of the sediment, an alternative site will be selected. The alternate site will be Carney Creek. The in-life study will not be initiated until analytical results confirming the LA contamination levels in the site sediment, or the lack of LA contamination in the lab control sediment or reference sediment. At exposure termination, sediment will be collected from each replicate tank, water decanted, and the sediment shipped to the SPF in Troy, MT for preparation prior to LA analysis by EMSL (Libby, MT)."

Page 9, Section 8.5.2. Sediment Analysis for LA

Test sediment (treatment group 3) will be analyzed for LA in accordance with the Libby-specific polarized light microscopy visual area estimation (PLM-VE) method by EMSL (Libby, MT). Sediment used for treatment group 3 will be collected in several "lots" by Remedium at TP-TOE2 and Carney Creek. Each lot will be well mixed prior to analysis. Sediment lots will be analyzed following the Libby-specific PLM-VE methods and the sediment lot(s) with concentration at 2% (or higher) submitted to FEL for testing. At the conclusion of the study, each replicate of the three treatments will be gently decanted to remove overlying and excess water. A sample of the wet sediment from each replicate will be collected in a glass bottle and submitted to the SPF in Troy, MT for preparation and subsequent analysis by EMSL (Libby, MT) to determine the final test exposure concentrations for treatment 3. Only sediment (treatment exposure medium) will be analyzed for LA in the present study (i.e., the overlying water will not be analyzed for LA).

Planned Change: At the conclusion of the exposure phase of study, sediment samples from the laboratory control (sand) and reference sediment will also be collected from each replicate and composited for LA analysis. All other specifications indicated above apply.

Reason for Change: Need to verify LA concentration in lab control and reference sediment.

Approval:

Study Director: _____ Date: 11/27/2012

Sponsor: _____ Date: 11/27/2012

QAU Manager: _____ Date: 11/27/2012

ATTACHMENT 2

Libby OU3 Amphibian Toxicity Study
On-site Evaluation Checklist (EPA only)

LIBBY OU3 AMPHIBIAN TOXICITY STUDY ON-SITE EVALUATION CHECKLIST

Date(s) of On-site: November 19, 2012

Laboratory: Fort Environmental Laboratories, Inc.

Address: 515 South Duncan Street

Stillwater, Oklahoma 74074

Telephone: (405) 624-6771

Personnel Contacted

Name	Title
Douglas J. Fort, Ph.D.	President
Michael B. Mathis	Quality Assurance Unit Manager (QAUM)
Genevieve M. Fent, Ph.D.	Laboratory Manager/Study Lead
Brittany Hall	Technician

Evaluation Team

Name	Title
Christina Progess	USEPA Region 8, Libby OU3 Project Manager
Michael P. Lenkauskas, CQA	Shaw Environmental, Senior Auditor
Sue Robinson	Golder Associates, Senior Toxicologist
Robert J. Medler	Remedium Group, Inc., Director

LIBBY OU3 AMPHIBIAN TOXICITY STUDY ON-SITE EVALUATION CHECKLIST

Date(s) of On-site: November 19, 2012

1.0 Facility & Equipment:	Yes	No	Comment
1.1 Is the facility secure (e.g., are all designated secure areas locked or have restricted access)?	☒	☐	Access to test/study facilities is limited to authorized personnel.
1.2 Is the laboratory workspace adequate?	☒	☐	
1.3 Is the toxicity test space adequate?	☒	☐	
1.4 Is laboratory water monitored for the following:	☒	☐	
1.4.1 pH (bimonthly)?	☒	☐	All bi-monthly and annual measurements are recorded in the applicable logbooks.
1.4.2 Dissolved Oxygen (bimonthly)?	☒	☐	
1.4.3 Conductivity (bimonthly)?	☒	☐	
1.4.4 Hardness (bimonthly)?	☒	☐	
1.4.5 Alkalinity (bimonthly)?	☒	☐	
1.4.6 Ammonia (bimonthly)?	☒	☐	
1.4.7 Residual Oxidants (bimonthly)?	☒	☐	
1.4.8 Iodide (annually)?	☒	☐	
1.4.9 PAHs (annually)?	☒	☐	
1.4.10 Pesticides (annually)?	☒	☐	
1.4.11 Metals (annually)?	☒	☐	
1.5 Do water quality measurements meet the USEPA and American Society for Testing (ASTM) criteria for aquatic toxicity test/culture water?	☒	☐	
1.6 Is analytical balance capable of weighing to 1 mg available?	☒	☐	The balance calibration logbook was reviewed for completeness.
1.6.1 Calibrated annually by certified technician?	☒	☐	
1.6.2 Calibrated daily (or when used) with Class S weights?	☒	☐	
1.7 Is refrigerator/freezer space adequate?	☒	☐	The refrigerator/freezer temperature logbooks were reviewed to ensure the documentation of daily monitoring.
1.7.1 Are refrigerator(s)/freezer(s) monitored daily to ensure they maintain the proper temperature(s)?	☒	☐	
1.8 Does the facility have adequate storage space?	☒	☐	
1.9 Is the facility maintained in a clean/organized manner?	☒	☐	

Additional Comments:

LIBBY OU3 AMPHIBIAN TOXICITY STUDY ON-SITE EVALUATION CHECKLIST

Date(s) of On-site: November 19, 2012

2.0 Toxicity Test Status
Provide a brief summary of the current status of the test. Include date initiated, projected termination date, problems encountered, deviations from protocol, corrective actions, and overall expectations.
The Test was initiated on September 26, 2012; the day of the on-site evaluation was Day 55 of the study. The test specimen (Southern Leopard Frog) stage of development at the time of the evaluation was approximately Gosner Stage 36, which was consistent across each of the exposure tanks: Exposure 1 (laboratory control sediment), Exposure 2 (field reference sediment), and Exposure 3 (Libby [Carney Creek] sediment). The test specimen are within days of metamorphism (Gosner Stage 46), at which time they will be removed from the exposure tanks and processed as described in the study protocol. To-date, there had been no observations of abnormal behavior, no specimen mortalities, and no deviations from the study protocol. Barring any unforeseen events or the potential for observations made during necropsy (if performed), the expected outcome of the study is that the specimen in Exposure 3 will show no adverse effects from exposure to the LA asbestos present in Exposure 3 and that behavior and growth across each of the exposure tanks was consistent.

LIBBY OU3 AMPHIBIAN TOXICITY STUDY ON-SITE EVALUATION CHECKLIST

Date(s) of On-site: November 19, 2012

3.0 Pre-test Activities	Yes	No	Comment
3.1 Test sediment, reference sediment and laboratory control QC			
3.1.1 Are the following analytical results available for the test sediment:			
3.1.1.1 LA by PLM-VE (list source)?	☒	☐	Analysis of the test sediment was performed by Remedium; however, FEL did have a copy of the results which were reviewed and found to be acceptable.
3.1.1.2 Pesticides by Method 8081A?	☒	☐	
3.1.1.3 Chlorinated Herbicides by Method 8151A?	☒	☐	
3.1.1.4 TOC by Method ASA 29-3?	☒	☐	
3.1.1.5 Diesel/Gasoline Range Organics by Method 8015B?	☒	☐	
3.1.1.6 Mercury by Method 7471A?	☒	☐	
3.1.1.7 Total Metals by Method 6010?	☒	☐	
3.1.1.8 Moisture Content by Method D2974?	☒	☐	
3.1.1.9 pH by Method ASA 10-3?	☒	☐	
3.1.1.10 Aroclors by Method 8082?	☒	☐	
3.1.1.11 Semivolatile Organics by Method 8270C?	☒	☐	
3.1.1.12 Acid Volatile Sulfide by Method AVS/TTR?	☒	☐	
3.1.1.13 Ammonia and N by Method ASA 33-7?	☒	☐	
3.1.2 Were analytical results found to be within acceptable range?	☒	☐	
3.1.3 Are the following analytical results available for the reference sediment and lab control (sand):			
3.1.3.1 LA by PLM-VE?	☒	☐	The results were available and reviewed.
3.1.3.2 Total Metals by Method 6010?	☒	☐	
3.1.3.3 PAHs?	☒	☐	
3.1.3.4 Aroclors by Method 8082?	☒	☐	
3.1.4 Were analytical results found to be within acceptable range?	☒	☐	
3.1.5 Were the analytical results confirming LA contamination levels in the site sediment, or lack of LA in the laboratory control and reference sediment, prior to the in-life study initiation (*Record results)*?	☐	☒	Confirmed in the test sediment at a concentration of approximately 5% LA, but not in either the laboratory control or the reference sediment. Refer to the summary report.
3.2 Study Specimens			
3.2.1 Test organisms selected for the study *(record)*	☒	☐	
3.2.2 Source of test organisms *(record)*	☒	☐	
3.2.3 Were all test organisms derived from the same clutch?	☒	☐	
3.2.4 Were test organism embryos held at 22-24ºC for 7 days to allow for hatching?	☒	☐	Approximately 4 days to reach Gosner Stage 20.

Additional Comments:

LIBBY OU3 AMPHIBIAN TOXICITY STUDY ON-SITE EVALUATION CHECKLIST

Date(s) of On-site: November 19, 2012

3.0 Pre-test Activities	Yes	No	Comment
3.2.5 After hatching, were test organisms held in the following conditions until they reach Gosner Stage 20:			
3.2.5.1 Separated to a density of 5-10 animals/L?	☒	☐	
3.2.5.2 At a constant flow rate of 12 mL/minute?	☒	☐	
3.2.5.3 Water temperature of 20-23ºC?	☒	☐	
3.2.6 Were test organisms (larvae) requiring >5 days post hatch to reach Gosner Stage 20 not used for the in-life test?	NA	NA	All test organisms reached Gosner Stage 20 in approximately 4 days.
3.2.7 If >50% of the larvae in a given clutch require >20 days to reach Gosner stage 20, was an alternate clutch used?	NA	NA	All test organisms reached Gosner Stage 20 in approximately 4 days.
3.2.8 Is the developmental stage of the animals determined using a binocular dissection microscope for examination of tail morphology?	☒	☐	
3.2.9 Once animals meet the Gosner Stage 20 criteria, are they transferred to a holding tank containing 100% dilution water until the staging process is completed?	☒	☐	

Additional Comments:

LIBBY OU3 AMPHIBIAN TOXICITY STUDY ON-SITE EVALUATION CHECKLIST

Date(s) of On-site: November 19, 2012

4.0 Staging Process	Yes	No	Comment
4.1 Exposure tanks:			
4.1.1 Glass aquaria (2.5 gallon)?	☒	☐	The exposure tanks were visually inspected.
4.1.2 Equipped with standpipes?	☒	☐	
4.1.3 Contain 2 cm of sediment (1.5 Kg)?	☒	☐	
4.1.4 Have minimum water depth of 25-30 cm (6 liters)?	☒	☐	
4.1.5 Material in contact with test water is glass, stainless steel or Teflon®?	☒	☐	
4.2 Equipped with fluorescent lighting to provide a photoperiod of 12 hours light and 12 hours dark at an intensity that ranges from 600 to 2,000 lux (lumens/m^2) at the water surface?	☒	☐	
4.3 Are each of the exposures set up accordingly and in quadruplicate:			
4.3.1 Exposure #1 – Laboratory control sediment comprised of inert sterilized sand and laboratory dilution water?	☒	☐	
4.3.2 Exposure #2 – Field reference sediment (collected by FEL from a reference pond in Oklahoma) of similar constitution as the test sediment and laboratory dilution water?	☒	☐	
4.3.3 Exposure #3 – Test sediment collected from Libby mine location containing approximately 2% LA and laboratory dilution water?	☒	☐	The test sediment contained approximately 5% LA.
4.4 Is the dilution water in each tank maintained at the following conditions:			
4.4.1 Water temperature at 20-23ºC?	☒	☐	The study records were reviewed to confirm conditions.
4.4.2 pH between 6.5 and 8.5?	☒	☐	
4.4.3 Dissolved oxygen (DO) concentration >3.5 mg/L?	☒	☐	
4.5 Is water renewed using a flow through system (12 L/min)?	☒	☐	
4.6 In order to provide sufficient aeration, is overlying water within each tank aerated using a micro-bubble diffuser (if needed)?	☒	☐	
4.7 Is the sediment/sand and water allowed to equilibrate for 24 hours prior the introduction of test organisms?	☒	☐	

Additional Comments:

LIBBY OU3 AMPHIBIAN TOXICITY STUDY ON-SITE EVALUATION CHECKLIST

Date(s) of On-site: November 19, 2012

5.0	In-Life Study	Yes	No	Comment
5.1	Once the staging has been completed, are larvae randomly distributed to exposure tanks until each tank contains 20 larvae?	☒	☐	
5.2	Are five randomly selected Gosner stage 20 pre-exposed tadpoles humanely euthanized in 150-200 mg/L MS-222 (pH 7), and preserved to verify stage upon in-life test setup?	☒	☐	Pre-exposed test specimen were examined.
5.3	Was each exposure tank initially inspected for animals with abnormal appearance (e.g., injuries, abnormal swimming behavior, etc.)?	☒	☐	None observed.
5.3.1	If yes, were overtly unhealthy tadpoles removed from the exposure tanks, and replaced with larvae newly selected from the pooling tank?	NA	NA	
5.4	Are tadpoles fed boiled organic romaine lettuce leaves (ad libitum), which are used in lieu of Sera Micron® (commonly used for Xenopus sp.), to minimize biofouling of the tank water?	☒	☐	
5.5	Are tanks cleaned using a turkey baster to remove organism detritus and excrement from the bottom of the tanks?	☒	☐	
5.6	Are each of the exposure takes monitored at the required frequency for the following:			
5.6.1	Temperature (daily)?	☒	☐	The study records were reviewed to confirm.
5.6.2	pH (3x a week)?	☒	☐	
5.6.3	Dissolved Oxygen (3x a week)?	☒	☐	
5.6.4	Light Intensity (3x a week)?	☒	☐	
5.7	Are the following measured in each exposure tank at the beginning, conclusion, and semi-monthly during the exposure stage:			
5.7.1	Total hardness?	☒	☐	The study records were reviewed to confirm.
5.7.2	Alkalinity?	☒	☐	
5.7.3	Conductivity?	☒	☐	
5.7.4	Total residual oxidants?	☒	☐	
5.7.5	Ammonia as N?	☒	☐	
5.8	Once all larvae have been assigned to an exposure tank, are mortality observations and developmental stage determinations made daily, any dead larvae immediately removed, preserved in 10% neutral buffered formalin (NBF), and necropsied?	☒	☐	Developmental stage determined daily. As of the date of the evaluation there were no mortalities.
5.9	Are all cases of abnormal behavior (e.g., uncoordinated swimming, hyperventilation, atypical quiescence, non-feeding, etc.) recorded?	NA	NA	None observed.

Additional Comments:

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Date(s) of On-site: November 19, 2012

6.0	Measurements at Metamorphosis	Yes	No	Comment
6.1	Upon metamorphosis, are tadpoles removed from the exposure tank anesthetized in 200 mg/L MS-222 (pH 7)?	NA	NA	At the time of the evaluation, none of the test specimen had reach metamorphosis.
6.2	Once anesthetized, are tadpoles rinsed in water, blotted dry, and weighed to the nearest mg?	NA	NA	
6.3	All SVL measurements (mm) based on digital photographs of the surviving organisms from each treatment?	NA	NA	
6.4	Is developmental stage data (Gosner) used to determine if development is accelerated, asynchronous, delayed, or unaffected? *Acceleration or delay of development is determined by making a comparison between the median stage achieved by the control and treated groups.* *Asynchronous development will be reported when the tissues examined are not malformed or abnormal, but the relative timing of the morphogenesis or development of different tissues is disrupted within a single tadpole.*	NA	NA	
6.5	Are external and internal (visceral) morphology evaluated in all surviving specimens at the conclusion of exposure to determine if exposure to LA is capable of inducing abnormal development? *External abnormalities will focus on eyes, mouth, torso, and hind limbs. Internal abnormalities will focus on primary organ systems, such as liver, kidneys, heart, and lung.*	NA	NA	
6.6	Are digital photographs of all abnormalities observed taken with the organism treatment designation and replicate number visible?			
6.7	Are head and carcass samples from the euthanized specimens fixed in Davidson's solution and preserved in 10% NBF for possible future histopathological examination of the thyroid gland and presumptive gonad tissue?	NA	NA	
6.8	Is plasma collected from MS-222 anesthetized specimens and stored frozen for possible future thyroid hormone measures (separate study)?	NA	NA	
6.9	Is the number of live tadpoles completing metamorphosis in each replicate of each treatment determined?	NA	NA	
6.10	Is the time to metamorphosis (TTM) in days determined for each individual frog and the median time to metamorphosis (MMT) determined for each replicate of each treatment or control?	NA	NA	

Additional Comments:

Although the elements of the termination process could not be verified at the time of the evaluation, FEL staff described the process, including a demonstration of the photo digitizing software that will be used to make test specimen measurements (i.e., Snout Vent length [SVL]).

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Date(s) of On-site: November 19, 2012

7.0 Test Termination
Since test termination is not scheduled to occur until after the on-site evaluation, interview laboratory personnel concerning anticipated difficulties and/or deviations from protocol.
Termination of the study is scheduled for no later than December 24, 2012. At the time of the evaluation, FEL staff indicated that no deviations to the study protocol are expected.

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Date(s) of On-site: November 19, 2012

8.0 Data Management	Yes	No	Comment
8.1 Are test data and daily observations recorded in the study records, including:			
8.1.1 Study tracking sheets?	☒	☐	
8.1.2 Test information sheets?	☒	☐	
8.1.3 Calendars identifying major events?	☒	☐	
8.1.4 Detailed observations and comments?	☒	☐	
8.1.5 Daily mortality and developmental stage data sheets?	☒	☐	
8.1.6 Test termination data sheets?	☒	☐	
8.1.7 Representative digital photographs taken during the conduct of the test.	☒	☐	
8.2 Are photographs used to document the study design, study milestones, endpoints (length measurements, development (external), abnormalities, and necropsy of the fully metamorphosed specimens?	☒	☐	
8.3 Are the number of organisms metamorphosed recorded as the time to metamorphosis (TTM) for each larvae, the weight of each newly metamorphosed larvae, and the median time to metamorphosis (MMT) determined when 50% of the larvae in a given replicate metamorphose?	NA	NA	At the time of the evaluation, none of the test specimens had reached metamorphosis.
8.4 Are the primary endpoints of the metamorphosis assay:			
8.4.1 Mortality?	NA	NA	Since none of the test specimens had reached metamorphosis at the time of the evaluation, this could not be confirmed, but staff did indicate these will be the primary endpoints.
8.4.2 Developmental stage (Gosner)?	NA	NA	
8.4.3 Number of specimens metamorphosed?	NA	NA	
8.4.4 Weight at metamorphosis?	NA	NA	
8.4.5 TTM and MMT for each replicate?	NA	NA	
8.4.6 Snout-vent length (SVL) with digital photographs?	NA	NA	
8.4.7 Wet whole body weight?	NA	NA	
8.4.8 External and internal abnormalities?	NA	NA	
8.4.9 Are personnel responsible for original data entry identified at the time of data input?	☒	☐	All entries are identified by date, time, and initials.

Additional Comments:

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Date(s) of On-site: November 19, 2012

9.0 Quality Assurance/Quality Control (QA/QC)	Yes	No	Comment
9.1 Has the Contractor established a QA/QC program that applies controls to the collection of data?	☒	☐	
9.2 What components of a QA/QC program have been implemented:			
9.2.1 Quality Assurance Plan (QAP)?	☒	☐	
9.2.2 Standard Operating Procedures (SOPs)?	☒	☐	
9.2.3 Internal QA Inspections?	☒	☐	
9.2.4 Processes to ensure adherence to method requirements?	☒	☐	
9.2.5 Processes to ensure documentation requirements are met?	☒	☐	
9.2.6 Processes for corrective action?	☒	☐	
9.3 Are QA documents, including QAP and SOPs, reviewed and approved annually by analysts, supervisor, and management?	☐	☐	
9.4 Was the study conducted in accordance with the specifications identified in the:			
9.4.1 Phase V-B Sampling and Analysis Plan (SAP)?	☐	☒	Study protocol needs to be revised to include PLM-VE analysis of laboratory and reference sediments upon termination. Refer to the summary report.
9.4.2 FEL Quality Assurance Management Plan (QAMP)?	☒	☐	
9.4.3 Relevant facility standard operating procedures (SOPs)?	☒	☐	
9.4.4 Study Protocol No. GOLD03-1 prepared for FEL Study No. GOLD03-0277?	☒	☐	
9.5 **Standard Operating Procedures:**			
9.5.1 Are the necessary written procedures available in the areas where the activities are performed?	☒	☐	

Additional Comments:
In addition to the above, the QAUM adheres to an inspection schedule that includes a protocol review; three Technical System Audits (TSAs) performed at the beginning, midpoint, and conclusion of the study; an analytical draft report review; and a review of the draft and final report.